

Laccase-catalysed coloration of wool and nylon

Chetna D. Prajapati,^a Edward Smith,^a Faith Kane^b and Jinsong Shen^{a,*} 

^aTextile Engineering and Materials (TEAM) Research Group, School of Design, De Montfort University, Leicester LE1 9BH, UK
Email: jshen@dmu.ac.uk

^bSchool of Design, College of Creative Arts, Massey University, Wellington 6140, New Zealand

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The potential for laccase (EC 1.10.3.2) to be used within the area of textile coloration, specifically for the generation of decorative surface pattern design, remains relatively unexplored. The current study presents a novel process for the coloration of wool and nylon 6,6 fibres via laccase oxidation of aromatic compounds as an alternative to conventional dyeing methods. Emphasis was placed on producing a diverse colour palette, which was achieved through the investigation of three different aromatic compounds as laccase substrates: 1,4-dihydroxybenzene, 2,7-dihydroxynaphthalene and 2,5-diaminobenzenesulphonic acid. Reaction processing parameters such as buffer systems and pH values, laccase and aromatic compound concentrations, and reaction times were investigated, all in the absence of additional chemical auxiliaries. Enzymatically dyed fabrics were tested against commercial standards, resulting in reasonably good colour fastness to wash. To demonstrate the coloration and design potential by laccase catalysation of aromatic compounds, specially constructed fabrics using a combination of undyed wool, nylon and polyester yarns were dyed using the one-step laccase-catalysed coloration process. The use of different fibre types and weave structures enabled simple colour variations to be produced. Shadow, reserve and contrasting effects were achieved with the laccase-catalysed dyeing process developed. Important advantages over conventional processing methods include the use of simpler and milder processing conditions that eliminate additional chemical use and reduce energy consumption.

Introduction

Coloration is an important process in textile finishing which is commonly used to enhance the appearance and attractiveness of a cloth. Conventional dyeing generally involves the use of several different chemicals, dyeing auxiliaries, in addition to elevated temperatures to assist the dyeing process. The coloration of wool and nylon can be achieved with the use of several dye classes, the most important of which are acid, mordant and premetallised dyes, all of which are applied under acidic conditions with the use of high temperatures, generally at the boil. The adoption of an alternative coloration approach using oxidative enzymes such as laccase could potentially offer processes with improved environmental sustainability by eliminating the inherent drawbacks associated with chemical processes [1,2].

Laccases (EC 1.10.3.2), belonging to a class of enzymes called oxidoreductase, can oxidise an extensive range of simple aromatic compounds such as diamines, aminophenols, aminonaphthols and phenols with or without a mediator, transforming them into coloured polymeric products via oxidative coupling reactions [3]. The reaction mechanism of laccase catalysation is a one electron oxidation of aromatic compounds to form free radicals while reducing molecular oxygen into water (Figure 1). These free radicals are very reactive and may go on to further react with the initial aromatic compound itself and polymerise in a non-enzymatic pathway to form coloured polymeric products [3,4]. These coloured oxidation products are capable of being adsorbed onto or reacting with numerous textile fibres for fibre coloration [5]. The use of laccase to synthesise new dyes or new synthesis

procedures of known textile dyes by enzymes has also been reported [6–8].

The versatility and capability by laccase of catalysing the oxidation of a broad substrate spectrum has led to a number of studies exploring the concept of laccase-assisted textile coloration of wool fibres [9–17]. The potential to dye other protein-based fibres such as hair has also been investigated [18,19]. Although an extensive list of laccase substrates are disclosed in patents and published papers, only a few substrates have been studied comprehensively. Current knowledge on hues achievable for wool coloration using laccase catalysis is limited to only a few mostly earthy tones. Previous studies have reported that variations of browns [9,10,13,15–17], greys [11], oranges [17], yellows [12] and purples [16] are achievable. Only a few studies have fully evaluated colour fastness properties of fabric dyed by laccase-catalysed coloration. Although studies have demonstrated that laccase-catalysed polymerisation of aromatic compounds can give rise to coloured products useful for dyeing wool, colour diversity reported is thus far too limited to be considered as a serious alternative to conventional acid and reactive dyes which offer a vast selection of hues ranging from bright to deep shades, and in some cases excellent colour fastness.

A broader survey of studies exploring laccase catalysis suggests greater colour diversity may be achievable with the use of numerous other known laccase substrates. Suparno *et al.* [20] reported that laccase oxidation of different dihydroxynaphthalenes resulted in purple, brown and green products. Polak and Jarosz-Wilkolazka [21], studying the transformation of benzene and naphthalene derivatives into dyes using fungal biomass (whole-cell biocatalysts), described an array of coloured products, including oranges,

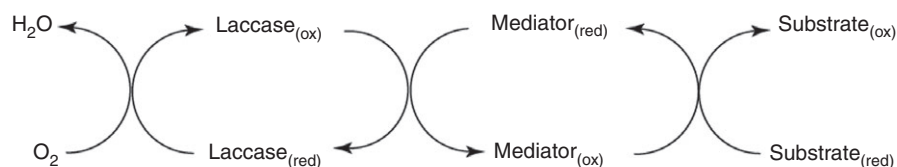


Figure 1 Laccase-catalysed oxidation of substrate through mediator [4]

reds, yellows, green/blues and purples. A similar range of coloured products were obtained by Sousa *et al.* [22] exploring the use of *p*-substituted primary aromatic amines for the synthesis of bio-colorants with laccase oxidation.

It is well known that enzymes have a characteristic optimal pH at which their activity is at a maximum; this can be either acidic, neutral or alkaline, and is often wider than one pH unit. The pH–activity relationship of any enzyme depends on the acid–base behaviour of both enzyme and substrate [23]. Shifts in pH values can lead to changes in the shape of the enzyme and affect electrostatic interactions within the enzyme, leading to a change in charge of the amino acids at the active site, with a subsequent possible impact on the enzyme activity towards the substrate. Studies concerned with the characteristics of laccase have reported that the surface charge of laccase can affect its catalytic activity towards its substrates, and that the optimal pH of laccase changes depending on the nature of both the enzyme and the substrate [24–26]. Past studies exploring the coloration potential of laccase have predominately favoured the use of an acetate buffer [10,11,13,17]. The use of other buffer systems has generally been overlooked.

The aim of this research was to develop a laccase catalysed *in-situ* dyeing process for wool and nylon fibres as an alternative to conventional methods. Emphasis was placed on producing a diverse colour palette through the exploration of different aromatic compounds as laccase substrates, in addition to reaction processing parameters: buffer systems and pH values, laccase and aromatic compound concentrations, and reaction times, all in the absence of additional chemical auxiliaries. Furthermore, enzymatically dyed fabrics would be tested against commercial standards to determine colour fastness properties. To investigate the use of enzymes for creative textile design, the results obtained from this investigation were further developed and applied to specially constructed jacquard weaves containing different fibre types and woven structures to generate decorative textile surface patterns. To date, no creative applications for laccase have been realised [27].

Experimental

Materials

Laccase (EC 1.10.3.2) with a measured activity of 800 LAMU/g was supplied by Novozymes A/S (Bagsvaerd, Denmark). Aromatic compounds including 1,4-dihydroxybenzene, 2,7-dihydroxynaphthalene and 2,5-diaminobenzenesulphonic acid were all purchased from Sigma-Aldrich (Dorset, UK), as illustrated in Table 1. Ultravon PL (UPL), a synergetic preparation based on nonionic surfactants, was purchased from Town End plc (Leeds, UK). All the other chemicals used were of specified laboratory reagent grade.

Undyed plain woven 100% wool fabric with a dry weight of 189 g/m², 50 ends per inch, 45 picks per inch, and a

Table 1 Structures of aromatic compounds selected as substrates for laccase-catalysed coloration

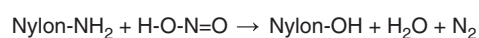
Aromatic compound	Acronym	Chemical structure
1,4-Dihydroxybenzene	1,4DHB	
2,7-Dihydroxynaphthalene	2,7DHN	
2,5-Diaminobenzenesulphonic acid	2,5DABS	

mean fibre diameter of 23 µm was supplied by Drummond Parkland (Huddersfield, UK). Undyed knitted single jersey 100% nylon 6,6 fabric with a dry weight of 159 g/m² was purchased from Ray Musson Knitting (Leicester, UK). Woven jacquard fabric samples were produced by Camira Fabrics (Mirfield, UK) constructed with 1200 warp ends using 100% undyed 2/20 Nm wool with 45 ends per inch and undyed weft yarns of nylon 6,6. Camira Fabrics also produced a range of weaves using wool of the same structure in the warp and undyed weft yarns of cotton, nylon 6,6, polyester (PET) or polyethylene.

Fabric preparation

Prior to any treatment, the wool fabric was scoured in a solution containing 1.6 g/l of sodium carbonate and 2 g/l UPL at 60 °C for 30 min, and the nylon fabric was scoured in a solution containing 2 g/l UPL at 70 °C for 30 min. Scouring for both fabric types took place in a Datacolor Ahiba Nuance IR dye machine at an agitation of 40 rpm using a liquor-to-goods ratio of 20:1. Scouring was followed by a hot and cold tap water rinse before air-drying at room temperature.

To study the effect of the amino end groups on dyeability, amino end groups were removed from nylon samples using the Van Slyke method as illustrated in Scheme 1, according to a procedure used by Smith [28].



Scheme 1 Removal of amino end groups from nylon using the Van Slyke method

An aqueous solution of nitrous acid was made by dissolving 0.5 g of sodium nitrite in 100 ml of deionised

water. The solution was acidified with 0.3 ml of acetic acid to form nitrous acid and adjusted to pH 4.0 using sodium acetate trihydrate. Pre-scoured nylon fabric (10 g) was then added to the solution and treated at 100 °C for 60 min. Treatment took place in a Datacolor Ahiba Nuance IR dye machine at an agitation of 40 rpm using a liquor-to-goods ratio of 10:1. After the treatment, the nylon fabric was rinsed in tap water and then left to air-dry at room temperature.

Enzymatic dyeing (one-step in-situ dyeing process)

Fabric samples (1 g) were thoroughly wetted out in deionised water before being placed in a bath containing 1% owf laccase and 1% omf or greater of a chosen aromatic compound. A liquor-to-goods ratio of 30:1 was used. To cover a range of pH values from pH 2.0–11.0, a range of different buffer systems were selected for study (Table 2). All buffer solutions were prepared at room temperature using a concentration of 0.1 M. Enzymatic dyeing was performed in a Datacolor Ahiba Nuance IR dye machine; the temperature was raised by 2.5 °C/min and maintained at a temperature of 50 °C for 1, 2, 4 or 8 h with an agitation speed of 40 rpm. Control samples for each compound containing no laccase, and no aromatic compound, were also processed for comparison. Once dyeing was complete, all samples were washed using a hot and cold water rinse and then left to air-dry at room temperature.

A post-soap wash was introduced after the dyeing step to remove any unfixed polymer residues and deactivate any laccase present on the surface of the fabric. Selected enzymatically dyed samples were washed in a solution containing 2 g/l UPL at 40 °C for 15 min with a liquor-to-goods ratio of 20:1 in a Datacolor Ahiba Nuance IR dye machine. Samples were then removed and washed using a hot and cold tap water rinse and then left to air-dry at room temperature. Fabric samples were then tested for initial colour permanence.

Colour measurement of dyed fabrics

A Datacolor SF600 Plus CT reflectance spectrophotometer with an aperture diameter of 6.6 mm was used to determine the colour values, colour strength and differences between enzymatically dyed fabric samples, represented by the CIE $L^*a^*b^*$ colour space system. Each sample was folded into four and measured four times. All values were measured and calculated under controlled conditions using Color-Tools QC software with the illuminant and observer conditions of D₆₅ and 10°, respectively. An average colour measurement was calculated from the data collected for each sample.

Colour differences between control sample and enzymatically dyed fabric samples are represented as ΔE and were calculated using Eqn 1:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

where ΔL^* , Δa^* , and Δb^* represent the differences between the corresponding units of each fabric sample.

Colour analysis of enzyme treatment liquor

To gain insights into the coloured products generated through laccase catalysis, coloration reactions using laccase and aromatic compounds were performed. Solutions were prepared by adding 0.01 g of laccase and 0.01 g of aromatic compound to 30 ml of 0.1 M citrate buffer adjusted to various pH values from pH 3.0–8.0. Solutions were then placed in a Datacolor Ahiba Nuance IR dye machine; the temperature was raised by 2.5 °C/min and maintained at 50 °C for 4 h with an agitation speed of 40 rpm. Once processing was complete, all coloured solutions were filtered using Whatman qualitative 1 filter paper. Coloured solutions obtained from laccase catalysation of 2,5DABS were then diluted; 1 ml of the coloured solution was added to 9 ml of citrate buffer which was adjusted to the resulting pH values of the coloured solutions. Solutions were then analysed using a Jasco Model V-570 UV/VIS/NIR spectrophotometer.

Colour fastness of enzyme-dyed fabrics

To determine colour fastness to domestic laundering, enzymatically dyed samples were tested in accordance with BS EN ISO 105-C10:2007 (Part C10: Colour fastness to washing with soap or soap and soda) using a Roaches Washtec washwheel. Grades obtained from colour fastness to washing were compared to Woolmark Quality Standards Specification AW-1: 2016 (with reference to Colour fastness to machine washing, Part A: standard detergent without perborate).

To determine the resistance of the colour of enzymatically dyed samples to rubbing off and staining other materials, tests were carried out in accordance with BS EN ISO 105-X12:2002 (Part X12: Colour fastness to rubbing). Fastness to rubbing under both dry and wet conditions was carried out using a crockmeter (James Heal, Halifax, UK).

To determine light fastness properties, enzymatically dyed samples were tested in accordance with BS EN ISO 105-B02:1999 (Part B02: Colour fastness to artificial light: Xenon arc fading lamp test). Samples were tested using Exposure Method 1 using a Q-SUN B02 Xenon Test Chamber. Exposure was conducted for a total of 270 h

Table 2 Range of buffer systems studied

Buffer system	Compounds used in composition	pH range
Hydrochloric acid/potassium chloride buffer	Hydrochloric acid and potassium chloride	2.0–4.0
Acetate buffer	Acetic acid and sodium acetate	3.0–7.0
Citrate buffer	Citric acid monohydrate and trisodium citrate	3.0–8.0
Phosphate buffer	Sodium phosphate dibasic dihydrate and sodium phosphate monobasic monohydrate	4.0–9.0
Bicarbonate/carbonate buffer	Sodium bicarbonate and sodium carbonate decahydrate	9.0–11.0

during two different periods: 80 h followed by a further 190 h. A set of three known controls, blue wool references 4, 5 and 6, were used during the test. A numerical rating was given to each sample: the number of the reference which showed a similar change in colour.

Results and Discussion

To investigate the use of laccase to catalyse the *in-situ* dyeing of wool and nylon 6,6 fabrics, a range of nine commercially available aromatic compounds were chosen as laccase substrates, principally because of their varying chemical structures. Laccase catalysis of each aromatic compound resulted in the synthesis of coloured compounds producing distinct colour shades in both liquor solution and on fabric [29]. Coloration of nylon fabric was notably lighter and brighter than on wool fabric. Because of the coloration potential observed with varying chemical structures, three of the aromatic compounds were selected for their results to be presented and investigated further (Table 1), namely 1,4-dihydroxybenzene (1,4DHB), 2,7-dihydroxynaphthalene (2,7DHN) and 2,5-diaminobenzene-sulphonic acid (2,5DABS).

Wool and nylon fabrics treated with laccase only in the absence of an aromatic compound led to no visible colour change after enzymatic treatment of both fibre types. Treatments containing an aromatic compound in the absence of laccase resulted in no colour to subtle colour changes across both fibre types, with the greatest difference observed on wool treated with compound 2,7DHN (Table 3). Residual liquor solutions in all cases remained clear and colourless after processing, with no visible evidence of colour formation. Both sets of control experiments confirmed the exclusion of either an aromatic compound or laccase from the treatment solutions resulted in neither colour formation in the liquor baths nor significant coloration of either fibre type.

Effect of pH and buffer systems on the activity of laccase towards aromatic compounds and the coloration of fibres

The pH value is of critical importance in order to optimise enzymatic catalysis. Past studies have predominantly favoured the use of an acetate buffer at pH 5.0 [10–17], while the use of alternative buffer systems and pH values

have been overlooked. The influence of different buffer systems and pH values on the *in-situ* coloration characteristics of the three aromatic compounds when used with laccase was investigated. To cover a range of pH values from pH 2.0–11.0, a range of different buffer systems were selected for study, as shown in Table 2.

Laccase and aromatic compound treatments resulted in a diverse range of characteristic hues and depths of shade being produced on both fibre types. Each aromatic compound gave rise to distinctive colour ranges, and the use of different buffer systems resulted in subtle differences in coloration characteristics across both wool and nylon fibres.

Compound 1,4DHB in the presence of laccase resulted in two contrasting hues being observed on each fibre. A variety of browns on wool and an assortment of pinks on nylon were observed, as shown in Table 4. The use of a citrate buffer produced a more diverse colour range in comparison to other buffer systems. In all cases wool fabric samples were darker than nylon, with deeper shades of browns on wool being produced with pH values of 6.0 and 7.0, and brighter pinks on nylon with pH values of 5.0 and 6.0.



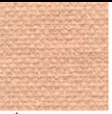











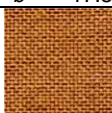
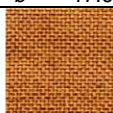
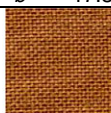


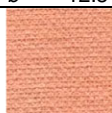
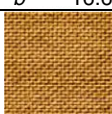
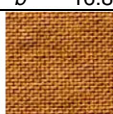
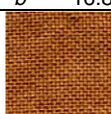


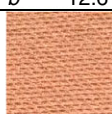

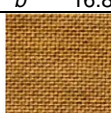


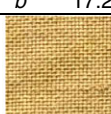

A variety of three distinctive hues, yellow, green and blue, were observed after enzymatic treatment with compound 2,7DHN across both fibre types (Table 5); pH values from 3.0 to 5.0 resulted in a variety of characteristic yellows; however, the use of pH ≥ 6.0 resulted in profound shifts in hue for both fibre types after enzymatic treatment. A variety of greens were produced with pH 6.0 and pH 8.0–11.0 for wool, and pH 6.0 for nylon. An assortment of blues was obtained with pH 7.0 for wool, and pH 7.0–11.0 for nylon. The strongest blues were produced using pH 7.0, of which the citrate and acetate buffers produced the brightest blues on both fibre types. The shifts in hues observed correlated with colour measurements recorded; on the whole a^* and b^* values shifted from positive to negative values. The results suggested that the use of treatment conditions between pH 6.0 and 11.0 promoted either the laccase, aromatic compound or radicals formed through laccase catalysis to react in very different ways to produce highly coloured products capable of generating hues which were previously unreported on wool and nylon fibres. Interestingly, coloration treatments carried out using pH values from 3.0–7.0 resulted in similar hues across both fibre types; however, pH 8.0–11.0 resulted in contrasting hues on each fibre type with greens on wool and blues on nylon. The greener hue observed on wool but not on nylon at \geq pH 8 may be caused by residual 2,7DHN uncatalysed by the enzyme, as when 2,7DHN was treated on wool and nylon (Table 3) a greenish tinge was observed on wool, but not on nylon.

Treatments with the compound 2,5DABS in the presence of laccase resulted in an array of brown, orange and yellow hues, ranging from deep to light shades across both fibre types (Table 6). Subtle variations in hues were observed across the range of buffer systems. The most effective pH ranges for dyeing wool with compound 2,5DABS were pH 3.0–8.0 for wool, and pH 3.0–6.0 for nylon. Coloration performed using acid conditions were found to be the most suitable for producing the deepest and/or brightest shades. Wool fabric samples were darker in comparison to nylon samples which were lighter and/or brighter. At pH 3.0 using a citrate buffer, a purple colour was observed on both wool

Table 3 $L^*a^*b^*$ results of wool and nylon after control studies with either laccase only or aromatic compound only

Enzyme treatment	pH	Wool				Nylon			
		L^*	a^*	b^*	ΔE	L^*	a^*	b^*	ΔE
No treatment	–	85.7	–0.0	9.7	–	93.7	–0.2	1.5	–
Laccase only	4	84.3	0.5	10.2	1.6	93.1	–0.0	2.9	1.6
	6	87.3	0.1	10.4	1.7	92.8	0.0	2.9	1.7
	8	85.2	0.7	10.2	1.0	92.5	0.2	2.2	1.5
2,5DABS only	4	80.2	2.0	11.9	6.3	88.8	2.2	4.3	6.2
1,4DHB only	6	77.7	2.8	11.7	8.8	88.8	5.1	4.1	7.7
2,7DHN only	8	68.8	–5.9	11.7	18.1	87.5	0.1	2.7	6.4

Table 4 Colour range achieved on wool and nylon when treated with 1,4DHB in the presence of laccase using different pH and buffer systems

pH	Wool			Nylon		
	AB	CB	PB	AB	CB	PB
3	 L^* 54.5 a^* 11.8 b^* 16.8	 L^* 52.4 a^* 11.1 b^* 17.7	-	 L^* 74.4 a^* 12.8 b^* 11.1	 L^* 76.9 a^* 5.2 b^* 13.9	-
4	 L^* 48.1 a^* 12.4 b^* 18.0	 L^* 46.2 a^* 12.4 b^* 17.5	-	 L^* 68.1 a^* 14.3 b^* 11.2	 L^* 70.5 a^* 12.1 b^* 10.6	-
5	 L^* 39.7 a^* 14.8 b^* 17.3	 L^* 41.5 a^* 14.7 b^* 17.6	 L^* 37.7 a^* 15.4 b^* 17.3	 L^* 68.7 a^* 19.1 b^* 12.4	 L^* 66.5 a^* 20.6 b^* 13.2	 L^* 66.1 a^* 22.0 b^* 12.5
6	 L^* 34.1 a^* 15.2 b^* 16.6	 L^* 37.4 a^* 15.4 b^* 16.8	 L^* 34.4 a^* 16.3 b^* 16.8	 L^* 66.4 a^* 19.4 b^* 13.4	 L^* 66.5 a^* 20.5 b^* 13.3	 L^* 66.1 a^* 21.8 b^* 12.6
7	 L^* 39.2 a^* 11.1 b^* 17.0	 L^* 35.9 a^* 13.8 b^* 16.7	 L^* 34.2 a^* 16.1 b^* 16.8	 L^* 62.4 a^* 15.5 b^* 12.9	 L^* 63.0 a^* 17.1 b^* 12.8	 L^* 58.8 a^* 18.4 b^* 12.4
8	-	 L^* 45.1 a^* 8.3 b^* 16.7	 L^* 37.9 a^* 11.2 b^* 17.2	-	 L^* 70.2 a^* 7.0 b^* 9.9	 L^* 72.0 a^* 8.3 b^* 10.0
9	-	-	 L^* 49.3 a^* 7.5 b^* 16.9	-	-	 L^* 73.4 a^* 3.6 b^* 5.7




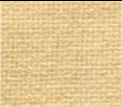


















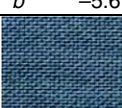
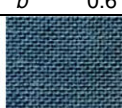
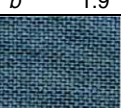
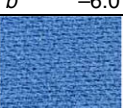

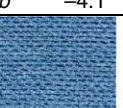
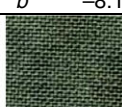
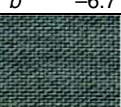
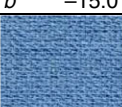

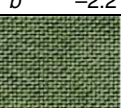

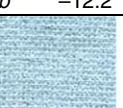

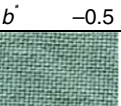
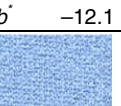
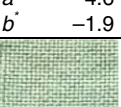
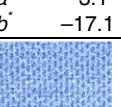
AB, acetate buffer; CB, citrate buffer; PB, phosphate buffer.

and nylon, but not with either acetate or hydrochloric buffers, which were orange-brown for wool and yellow-orange for nylon. In general, at pH 4.0–6.0 for all buffer systems, wool was coloured orange-brown; but nylon was coloured yellow-orange at pH 4.0–5.0 and a much paler shade of yellow-orange at pH 6.0. As treatment buffer conditions moved towards neutral and alkali conditions, the coloration of both fibre types either gradually became lighter, and/or ineffective with no uptake of coloured products synthesised *in-situ*. Therefore, pH > 6 was not suitable for coloration of nylon to take place.

It is believed that the coloration created with all compounds on both fibre types was a result of laccase





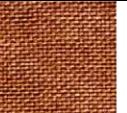




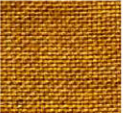
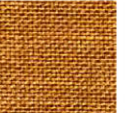
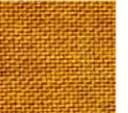




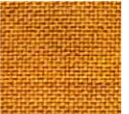
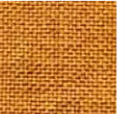
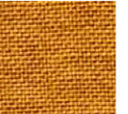


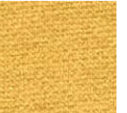





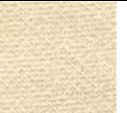




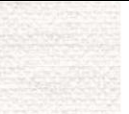







oxidative coupling facilitated by the action of laccase, which is able to extract hydrogen protons from substitute groups, $-\text{NH}_2$, $-\text{OH}$, $-\text{COOH}$ present in aromatic amino acid residues of wool like tyrosine. One-electron oxidation of hydroxylated aromatic substrates is accompanied by the reduction of molecular oxygen to water by the transfer of electrons, resulting in highly reactive free radicals being produced capable of reacting non-enzymatically to create coloured products of different polymeric molecular weights. It could be possible for wool to be directly involved in the enzymatic polymerisation of colorants through the aromatic amino acid residues (tyrosine) of wool. Non-enzymatic reaction

Table 5 Colour range achieved on wool and nylon when treated with 2,7DHN in the presence of laccase using different pH and buffer systems

pH	Wool				Nylon			
	AB	CB	PB	BCB	AB	CB	PB	BCB
3	 L^* 77.3 a^* -0.4 b^* 12.2	 L^* 66.7 a^* 1.0 b^* 20.6	-	-	 L^* 87.2 a^* 2.0 b^* 5.7	 L^* 77.6 a^* 3.7 b^* 15.5	-	-
4	 L^* 59.9 a^* 0.9 b^* 17.3	 L^* 60.3 a^* 1.5 b^* 18.1	 L^* 52.0 a^* 2.6 b^* 13.2	-	 L^* 75.2 a^* 3.9 b^* 12.8	 L^* 67.8 a^* 6.4 b^* 22.0	 L^* 64.9 a^* 5.8 b^* 19.6	-
5	 L^* 47.5 a^* 0.9 b^* 10.1	 L^* 48.6 a^* 2.3 b^* 12.8	 L^* 44.2 a^* 2.0 b^* 10.8	-	 L^* 57.9 a^* 4.3 b^* 15.2	 L^* 57.7 a^* 4.3 b^* 14.3	 L^* 57.2 a^* 4.4 b^* 14.8	-
6	 L^* 30.6 a^* -4.3 b^* -5.6	 L^* 33.8 a^* -2.6 b^* 0.6	 L^* 36.4 a^* -1.8 b^* 1.9	-	 L^* 40.0 a^* -3.5 b^* -6.0	 L^* 40.1 a^* -2.7 b^* -4.0	 L^* 40.8 a^* -2.2 b^* -4.1	-
7	 L^* 27.2 a^* -4.2 b^* -7.9	 L^* 27.4 a^* -4.1 b^* -8.1	 L^* 28.6 a^* -3.6 b^* -6.7	-	 L^* 36.7 a^* -2.5 b^* -16.4	 L^* 37.0 a^* -2.5 b^* -15.0	 L^* 37.2 a^* -2.6 b^* -12.6	-
8	-	 L^* 25.6 a^* -2.1 b^* 2.6	 L^* 27.3 a^* -3.4 b^* -2.2	-	-	 L^* 39.3 a^* -2.3 b^* -12.9	 L^* 40.3 a^* -2.4 b^* -12.2	-
9	-	-	 L^* 34.1 a^* -3.3 b^* 4.5	 L^* 36.5 a^* -5.0 b^* -0.5	-	-	 L^* 56.2 a^* -4.7 b^* -7.2	 L^* 53.9 a^* -5.0 b^* -12.1
10	-	-	-	 L^* 43.7 a^* -4.6 b^* -1.9	-	-	-	 L^* 58.8 a^* -3.1 b^* -17.1
11	-	-	-	 L^* 53.4 a^* -4.4 b^* 4.2	-	-	-	 L^* 55.9 a^* -1.4 b^* -17.8








AB, acetate buffer; CB, citrate buffer; PB, phosphate buffer; BCB, bicarbonate/carbonate buffer.

Table 6 Colour range achieved on wool and nylon when treated with 2,5DABS in the presence of laccase using different pH and buffer systems

pH	Wool				Nylon			
	CLB	AB	CB	PB	CLB	AB	CB	PB
2	 L^* 80.2 a^* 1.8 b^* 14.1	-	-	-	 L^* 89.3 a^* 0.90 b^* 4.29	-	-	-
3	 L^* 52.1 a^* 13.9 b^* 25.1	 L^* 37.3 a^* 19.8 b^* 29.2	 L^* 40.1 a^* 18.4 b^* 8.0	-	 L^* 67.7 a^* 8.0 b^* 18.6	 L^* 57.8 a^* 13.1 b^* 22.9	 L^* 70.0 a^* 10.0 b^* 4.9	-
4	 L^* 57.7 a^* 13.0 b^* 26.5	 L^* 43.6 a^* 17.9 b^* 32.8	 L^* 45.4 a^* 18.2 b^* 20.5	 L^* 38.8 a^* 15.8 b^* 23.4	 L^* 80.4 a^* 4.8 b^* 23.8	 L^* 69.9 a^* 17.2 b^* 37.3	 L^* 76.5 a^* 15.8 b^* 29.5	 L^* 65.2 a^* 14.1 b^* 32.4
5	-	 L^* 43.9 a^* 17.2 b^* 28.2	 L^* 42.2 a^* 15.3 b^* 19.8	 L^* 40.0 a^* 15.8 b^* 22.3	-	 L^* 75.4 a^* 9.2 b^* 24.6	 L^* 77.8 a^* 8.6 b^* 25.3	 L^* 73.6 a^* 10.4 b^* 26.8
6	-	 L^* 57.2 a^* 13.7 b^* 28.1	 L^* 51.0 a^* 14.2 b^* 22.0	 L^* 49.5 a^* 15.5 b^* 23.6	-	 L^* 86.5 a^* 2.1 b^* 16.0	 L^* 87.2 a^* 1.5 b^* 16.4	 L^* 85.7 a^* 3.8 b^* 13.6
7	-	 L^* 63.1 a^* 10.4 b^* 24.8	 L^* 61.4 a^* 10.0 b^* 24.7	 L^* 57.6 a^* 12.4 b^* 20.7	-	 L^* 89.4 a^* 0.7 b^* 10.6	 L^* 89.0 a^* 0.8 b^* 6.2	 L^* 90.2 a^* 0.8 b^* 4.7
8	-	-	 L^* 67.5 a^* 5.2 b^* 18.9	 L^* 69.0 a^* 5.1 b^* 17.2	-	-	 L^* 90.3 a^* 0.4 b^* 4.1	 L^* 90.6 a^* 0.1 b^* 2.8
9	-	-	-	 L^* 80.1 a^* 1.1 b^* 12.9	-	-	-	 L^* 90.6 a^* 0.3 b^* 2.9

CLB, hydrochloric acid/potassium chloride buffer; AB, acetate buffer; CB, citrate buffer; PB, phosphate buffer.

Table 7 Colour generated by laccase catalysis of 2,5DABS in solution using a citrate buffer set at different pH values

Sample	pH					
	3	4	5	6	7	8
Neat						
Diluted						

may also be involved in covalent bonding between laccase-catalysed radicals and amino groups found in wool and nylon fibres during laccase-catalysed coloration [3].

On the basis of the results obtained from investigations into buffer systems and pH values, the citrate buffer was chosen for further investigation primarily due to its wide pH range in comparison with other buffers. In addition, the citrate buffer offered the possibility of producing a more diverse colour palette, as observed from laccase catalysis of compounds studied. Treatments with 1,4DHB enabled greater coloration diversity on nylon, and with compound 2,7DHN offered the possibility of producing a brighter range of blues to strong greens simply through pH control. Furthermore, purple tones as well as strong shades of brown on wool and bright yellow-oranges on nylon were observed with compound 2,5DABS.

Analysis of laccase-catalysed colour in enzyme treatment liquors without fabrics

To gain insights into the coloured products generated through laccase catalysis, reactions were performed in the absence of fabric. Laccase catalysis of compound 2,5DABS produced coloured products of very high intensity (Table 7). Ultraviolet-visible (UV-vis) spectrum analysis of coloured products displayed a range of absorption peaks mainly at two wavelengths: a peak around 465 nm was observed which gradually disappeared with a decrease in pH, while another peak appeared at around 545 nm with an increase in pH (Figure 2). Treatments undertaken at pH 3.0 and 4.0 both produced pink coloured products, with the latter more intense than the former with an absorption value of 0.95. Although pH 4.0 was more effective at yielding higher coloured product quantities, stronger coloration on wool fibre type resulted from the use of pH 3.0 (Table 6). These results suggest dyeing at pH 3.0 could be

considered, where the coloured products formed may have greater affinity towards both fibre types. The use of pH 5.0 and 6.0 exhibited double peaks around 455–465 nm and 520–540 nm, respectively, strongly suggesting that the coloured products generated may consist of coloured mixtures similar to the ones generated with the use of pH 7.0–8.0 and 3.0–4.0, respectively. This indicated that the use of different pH conditions could effectively produce compounds of different structures imparting different coloured hues in solution and on fibre. In addition, coloured solutions resulting from reactions were observed to be pH-sensitive. The alteration of the solution pH after processing resulted in solutions shifting hue. This observation suggested coloured solutions may be altered post-processing. Zhang *et al.* [16] found that coloured solutions formed through the laccase catalysis of 2,5DABS with similar absorption characteristics as observed in this study could be converted by simply altering the pH conditions, leading to reversible coloured solutions and dyed wool fabrics. Although distinctive coloured solutions, primarily reds, oranges and yellows, resulted from reactions, this diversity did not transcend onto the coloration of wool and nylon fibres. Instead, a limited colour range was observed on both fibre types, mainly variations of red-browns to yellows, as shown in Table 6.

Effect of aromatic compound concentration

The effect of the concentration of laccase to aromatic compound was investigated to understand how coloration was affected. Laccase concentration was kept constant, but three different concentrations of the aromatic compound were used. Increasing the concentration of compounds 1,4DHB and 2,7DHN increased the depth of shade, similar to conventional dyeing methods, where an increase in dye concentration generates a higher depth of shade. However,

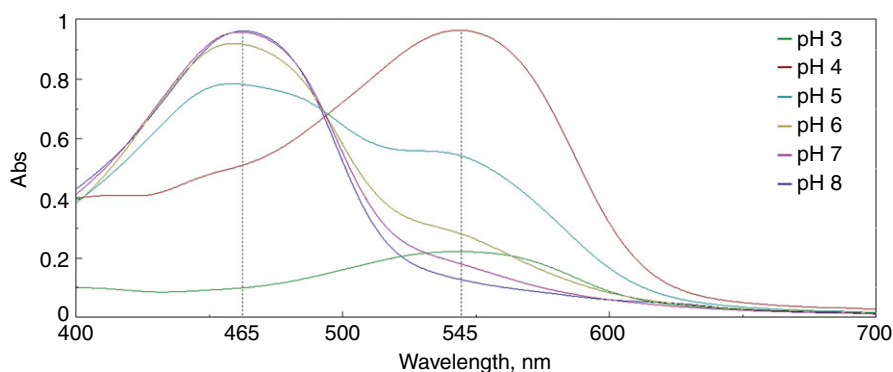
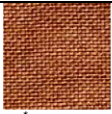
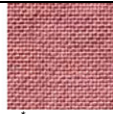
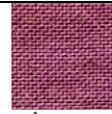


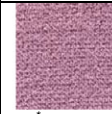
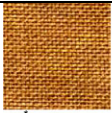
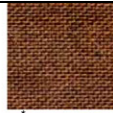





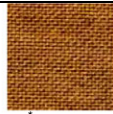

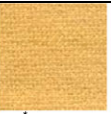



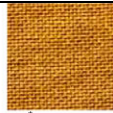
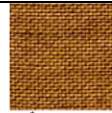



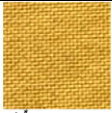
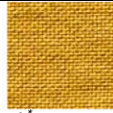
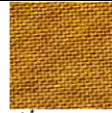



**Figure 2** UV-vis spectrum of coloured solutions generated by laccase catalysis of 2,5DABS using a citrate buffer set at different pH values

Table 8 Colour range achieved on wool and nylon when treated in the presence of laccase in a citrate buffer at different pH using varying concentrations of 2,5DABS

pH	Wool			Nylon		
	1:1	1:2	1:4	1:1	1:2	1:4
3	 L^* 40.1 a^* 18.4 b^* 8.0	 L^* 43.1 a^* 16.0 b^* 2.7	 L^* 33.5 a^* 16.6 b^* -2.6	 L^* 70.0 a^* 10.0 b^* 4.9	 L^* 49.5 a^* 10.8 b^* 1.3	 L^* 44.6 a^* 12.7 b^* -2.9
4	 L^* 45.4 a^* 18.2 b^* 20.5	 L^* 22.2 a^* 13.5 b^* 9.7	 L^* 16.2 a^* 8.3 b^* 2.3	 L^* 76.5 a^* 15.8 b^* 29.5	 L^* 58.6 a^* 16.5 b^* 25.0	 L^* 54.2 a^* 16.3 b^* 22.2
5	 L^* 42.2 a^* 15.3 b^* 19.8	 L^* 29.8 a^* 15.2 b^* 17.1	 L^* 21.9 a^* 13.0 b^* 10.2	 L^* 77.8 a^* 8.6 b^* 25.3	 L^* 68.0 a^* 17.2 b^* 32.3	 L^* 64.1 a^* 15.3 b^* 33.1
6	 L^* 51.0 a^* 14.2 b^* 22.0	 L^* 40.3 a^* 15.3 b^* 21.6	 L^* 31.0 a^* 14.4 b^* 18.0	 L^* 87.2 a^* 1.5 b^* 16.4	 L^* 83.0 a^* 7.4 b^* 27.1	 L^* 79.5 a^* 8.7 b^* 32.5
7	 L^* 61.4 a^* 10.0 b^* 24.7	 L^* 49.2 a^* 13.2 b^* 25.6	 L^* 39.2 a^* 13.2 b^* 20.3	 L^* 89.0 a^* 0.8 b^* 6.2	 L^* 88.8 a^* 1.6 b^* 12.7	 L^* 86.0 a^* 3.2 b^* 20.4

increasing concentrations of compound 2,5DABS with altering pH treatment conditions resulted in a remarkable array of different hues being observed, as shown in Table 8. These observations with 2,5DABS have not been previously reported. In particular, the use of pH 3.0 displayed two distinctive hues, an increase in compound concentrations enabled a shift in hue resulting in brown and purple coloration on wool. Similarly, the use of a 1:4 ratio of laccase-to-compound enabled multiple colours to be produced by simply altering the pH conditions. A purple was produced with pH 3.0, a dark brown with pH 4.0, and a variety of brown-yellows with pH 5.0–7.0 in the case of wool. A similar trend was observed with nylon, although the colours produced were lighter and brighter. The change in colour characteristics resulted in a change with b^* values shifting from blue (negative) to yellow (positive), representative of the hue shift observed on both fibres. These results suggest the presence of higher concentrations of 2,5DABS may enhance polymerisation possibilities, enabling radicals formed through catalysis to react in different ways. An increase in pH beyond pH 4.0 resulted in lighter coloration on both fibre types. The hues achieved on wool at 1:4 laccase-to-2,5DABS were more distinctive and more closely resembled the liquor colours, as illustrated in Table 7.

Effects of treatment time

The effect on coloration due to duration of laccase-catalysed *in-situ* dyeing was investigated. The results for 1,4DHB (Table 9) show that both wool and nylon displayed an increase in depth of shade with increasing reaction times; therefore, samples treated for 8 h with higher concentrations of 1,4DHB gave the deepest shades, with L^* values of 21.4 for wool and 54.2 for nylon. Longer treatment times suggested a deeper colour could simply be achieved by prolonging the contact time between laccase, aromatic compound and fibre. This is in contrast to conventional dyeing methods in which the depth of shade is proportional to the amount of dye present in the dye bath. However, it is worth noting that increasing the contact time from 4 to 8 h only marginally increased the depth of shade achieved, and therefore cannot be seen as economically beneficial.

Increasing treatment times from 1 to 2 h during the coloration of wool with 2,7DHN resulted in a deeper shade being produced with all ratios on wool; however, further increasing treatment times from 2 to 8 h resulted in no significant change (Table 10). In comparison, prolonging treatment times from 1 to 8 h with nylon resulted in a gradual increase in the depth of shade being produced with

Table 9 Colour range achieved on wool and nylon when treated in the presence of laccase at pH 6 using varying concentrations of 1,4DHB and duration of treatment






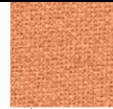
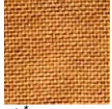

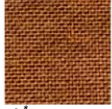

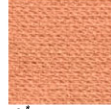
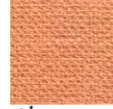
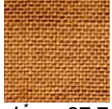





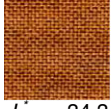
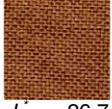
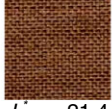
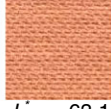


Time, h	Wool			Nylon		
	1:1	1:2	1:4	1:1	1:2	1:4
1	 L^* 46.9 a^* 15.8 b^* 17.4	 L^* 39.2 a^* 16.7 b^* 17.1	 L^* 33.5 a^* 16.2 b^* 15.5	 L^* 72.6 a^* 19.7 b^* 11.5	 L^* 68.9 a^* 21.4 b^* 12.9	 L^* 63.8 a^* 21.6 b^* 14.1
2	 L^* 41.5 a^* 15.9 b^* 17.0	 L^* 33.6 a^* 16.6 b^* 16.1	 L^* 27.1 a^* 15.6 b^* 13.5	 L^* 69.6 a^* 20.4 b^* 11.9	 L^* 65.5 a^* 22.3 b^* 13.5	 L^* 61.2 a^* 21.3 b^* 14.1
4	 L^* 37.7 a^* 15.7 b^* 16.8	 L^* 30.5 a^* 16.0 b^* 15.0	 L^* 23.6 a^* 14.5 b^* 11.5	 L^* 66.1 a^* 21.3 b^* 12.5	 L^* 61.8 a^* 22.3 b^* 14.0	 L^* 58.4 a^* 20.7 b^* 14.4
8	 L^* 34.3 a^* 15.3 b^* 15.6	 L^* 26.7 a^* 15.1 b^* 13.2	 L^* 21.4 a^* 12.9 b^* 9.3	 L^* 63.1 a^* 21.7 b^* 13.4	 L^* 60.3 a^* 20.3 b^* 13.4	 L^* 54.2 a^* 18.7 b^* 14.1

Table 10 Colour range achieved on wool and nylon when treated in the presence of laccase at pH 8 using varying concentrations of 2,7DHN and duration of treatment


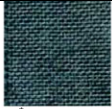
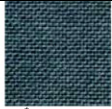
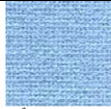
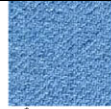
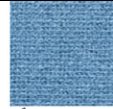
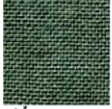
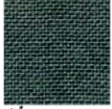
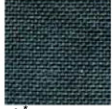
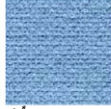
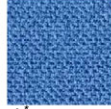
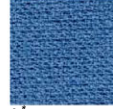
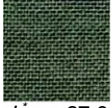




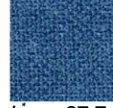
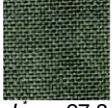
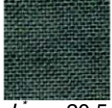

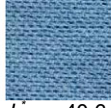
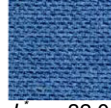

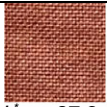
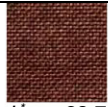



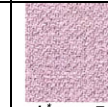

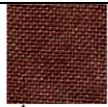



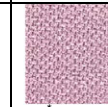




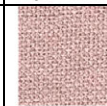
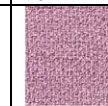




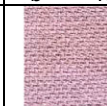

Time, h	Wool			Nylon		
	1:1	1:2	1:4	1:1	1:2	1:4
1	 L^* 31.2 a^* -4.7 b^* 1.2	 L^* 22.9 a^* -4.9 b^* -2.9	 L^* 21.9 a^* -5.6 b^* -3.8	 L^* 52.3 a^* -3.3 b^* -10.9	 L^* 43.3 a^* -3.3 b^* -14.2	 L^* 42.6 a^* -3.2 b^* -12.2
2	 L^* 28.4 a^* -3.6 b^* 2.1	 L^* 20.6 a^* -3.6 b^* -2.1	 L^* 16.9 a^* -2.6 b^* -3.0	 L^* 46.4 a^* -3.4 b^* -11.0	 L^* 36.2 a^* -3.0 b^* -15.26	 L^* 32.9 a^* -2.9 b^* -13.9
4	 L^* 27.6 a^* -3.3 b^* 2.0	 L^* 21.1 a^* -3.3 b^* -1.9	 L^* 16.5 a^* -2.0 b^* -2.5	 L^* 44.7 a^* -3.3 b^* -11.2	 L^* 33.1 a^* -2.4 b^* -15.5	 L^* 27.7 a^* -1.9 b^* -13.8
8	 L^* 27.3 a^* -2.8 b^* 2.4	 L^* 20.5 a^* -2.8 b^* -1.6	 L^* 16.1 a^* -1.4 b^* -2.4	 L^* 40.6 a^* -2.8 b^* -10.6	 L^* 32.0 a^* -2.4 b^* -14.4	 L^* 24.3 a^* -1.6 b^* -12.0

Table 11 Colour range achieved on wool and nylon when treated in the presence of laccase at pH 3 using varying concentrations of 2,5DABS and duration of treatment

Time, h	Wool			Nylon		
	1:1	1:2	1:4	1:1	1:2	1:4
1	 L^* 37.2 a^* 16.7 b^* 6.1	 L^* 23.7 a^* 15.0 b^* 4.6	 L^* 24.6 a^* 12.6 b^* -0.6	 L^* 68.3 a^* 5.3 b^* 4.5	 L^* 61.5 a^* 6.9 b^* 1.5	 L^* 52.8 a^* 8.9 b^* -3.3
2	 L^* 35.4 a^* 17.1 b^* 5.1	 L^* 21.7 a^* 15.4 b^* 3.4	 L^* 21.5 a^* 13.1 b^* -1.1	 L^* 64.9 a^* 6.2 b^* 5.7	 L^* 56.9 a^* 7.7 b^* 2.2	 L^* 49.3 a^* 10.2 b^* -2.0
4	 L^* 36.9 a^* 17.1 b^* 7.7	 L^* 22.1 a^* 15.4 b^* 4.2	 L^* 20.8 a^* 13.5 b^* -0.5	 L^* 61.1 a^* 7.1 b^* 4.7	 L^* 53.4 a^* 8.8 b^* 1.4	 L^* 45.8 a^* 11.5 b^* -2.7
8	 L^* 30.6 a^* 17.0 b^* 5.8	 L^* 20.1 a^* 13.7 b^* 2.9	 L^* 16.2 a^* 8.4 b^* 0.3	 L^* 59.6 a^* 9.9 b^* 3.2	 L^* 49.4 a^* 11.8 b^* -0.9	 L^* 45.0 a^* 13.8 b^* -4.4

all ratios. In general, a^* and b^* values remained constant; L^* values continued decreasing over time, with the greatest differences observed with ratio 1:4. As observed in earlier experiments, the coloration of wool and nylon remained consistently greener and bluer, respectively, with the use of pH 8.0.

Two pH values were chosen for investigation with compound 2,5DABS. The results for pH 3.0 are shown in Table 11. Increasing treatment times from 1 to 8 h during the coloration of wool resulted in no significant change with any of the considered ratios. In the case of nylon, a gradual depth in colour was observed with prolonged treatment times.

With pH 4.0, increasing treatment times from 1 to 4 h during the coloration of wool resulted in subtle colour characteristic shifts towards deeper shades being produced with all ratios; these were more obvious with ratio 1:1 than 1:4. However, increasing treatment times from 4 to 8 h resulted in no significant change (Table 12). Nylon coloration using the same conditions resulted in slight increases in depths of shade over time, with subtle colour shifts from pale yellow-oranges developing into medium shades of orange. Previous studies have reported that higher molecular weight compounds may be generated after longer reaction times [3,30]; this may explain why deeper shades resulted from longer reaction times in some cases.

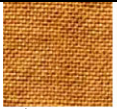




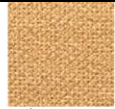
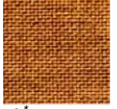
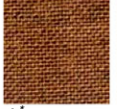
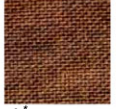
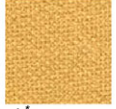
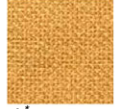
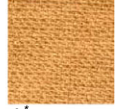






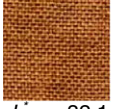
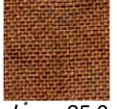
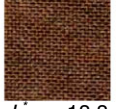

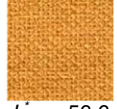

Colour fastness

Dyed samples were tested to ISO standards for colour fastness and staining due to washing. Different wash fastness values were observed on both fibre types (Table 13). In general, post-washing both fibre types prior to testing had little effect on performance, and in all cases nylon performed better than

wool. Visible changes in colour characteristics after testing were observed across all dyed fabrics, with the exception of nylon samples dyed with 1,4DHB. Both fibre types dyed with 1,4DHB resulted in no staining on the adjacent multifibre strips, with all tests obtaining a grade 5. In contrast, profound colour changes were observed with tests dyed with compound 2,5DABS, with wool samples achieving 1/2 and nylon achieving 3/4 grades. Although a lot of colour was lost to residual liquor, only subtle staining was observed on the cotton component of the multifibre strip for wool tests' all other fibre components remained unaffected. Tests performed with samples dyed with 2,7DHN resulted in greater cross-staining across the multifibre strip; only polyester and acrylic components remained unaffected. Only wool samples dyed with 1,4DHB matched the change in colour requirements and exceeded the standards for minimum staining requirements as stated in specifications AW-1: 2016 [31].

To evaluate the transfer of surface dye from the dyed test samples, rub fastness tests were conducted. Laccase dyeing with compounds 1,4DHB and 2,5DABS resulted in excellent rub fast results, with no staining being observed on either wool or nylon fibres during wet and dry test conditions (Table 13). However, tests conducted on samples dyed by laccase with compound 2,7DHN resulted in colour being transferred onto the cotton rubbing cloth during wet conditions, which was more prevalent on unwashed wool samples. On nylon, staining only occurred on the sample given no post-soaping, suggesting a post-soap wash at 40 °C may be necessary to remove residue dye from the surface of the fabric. No staining was observed using dry conditions for this compound. Grades obtained on dyed wool samples were checked against Woolmark Quality Standard specifications, and all compounds met the

Table 12 Colour range achieved on wool and nylon when treated in the presence of laccase at pH 4 using varying concentrations of 2,5DABS and duration of treatment

Time, h	Wool			Nylon		
	1:1	1:2	1:4	1:1	1:2	1:4
1						
	L^* 39.1 a^* 15.8 b^* 18.0	L^* 30.0 a^* 16.1 b^* 13.7	L^* 22.0 a^* 12.4 b^* 6.5	L^* 70.7 a^* 7.7 b^* 18.0	L^* 63.9 a^* 9.6 b^* 19.1	L^* 57.4 a^* 8.2 b^* 14.5
2						
	L^* 35.0 a^* 15.6 b^* 15.4	L^* 26.4 a^* 14.9 b^* 11.6	L^* 22.4 a^* 13.3 b^* 8.3	L^* 68.9 a^* 10.9 b^* 22.1	L^* 61.2 a^* 13.0 b^* 21.6	L^* 51.9 a^* 12.0 b^* 17.3
4						
	L^* 31.7 a^* 14.9 b^* 14.0	L^* 24.1 a^* 14.6 b^* 10.7	L^* 19.4 a^* 12.1 b^* 6.8	L^* 64.4 a^* 13.0 b^* 22.8	L^* 57.7 a^* 15.6 b^* 22.5	L^* 51.5 a^* 14.9 b^* 18.9
8						
	L^* 32.1 a^* 15.7 b^* 14.3	L^* 25.0 a^* 15.1 b^* 12.1	L^* 18.8 a^* 11.6 b^* 6.2	L^* 63.8 a^* 14.7 b^* 23.3	L^* 56.6 a^* 17.4 b^* 22.8	L^* 47.6 a^* 17.2 b^* 18.2

minimum standards required for colour fastness to rubbing (dry: AW-1: 2016 [31]; and wet: IF-1: 2016 [32]).

In this study, dyed samples were tested to commercial standards for colour fastness to light. Different light fastness levels were recorded for both fibres; however, none of the tested samples met the equivalent blue wool reference 4. A change in colour characteristics was observed across all dyed wool samples. Compounds 1,4DHB and 2,5DABS resulted in a loss of depth of colour. With compound 2,7DHN, a loss in depth of colour and a change in hue were observed. In contrast, profound fading, similar to a bleaching effect, was observed on all dyed nylon samples. Poor

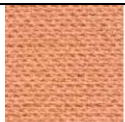

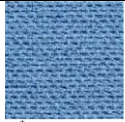
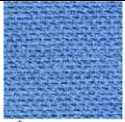
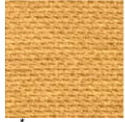

light fastness results suggest that coloured compounds responsible for coloration may consist of chemical structures which lack stable electron arrangements and are therefore susceptible to photo-oxidation. Furthermore, different levels of fading were observed across both fibre sets, indicating that coloured polymers may be bound to different functional groups present in each fibre type. This suggests that the chemical structure of fibres play an important role in light fastness properties. Grades obtained from colour fastness to light did not meet the minimum standards stated in specification AW-1: 2016 [31]. Most of the previous studies exploring the application of

Table 13 Colour fastness properties of enzymatically dyed wool and nylon fabrics

Compound	Fibre	Post-soap wash	Fastness to rub			Fastness to washing					
			Wet	Dry	Colour change	CA	CO	PA	PES	PAC	WO
1,4DHB	Wool	No wash	5	5	3/4	5	5	5	5	5	5
		40 °C	5	5	3/4	5	5	5	5	5	5
	Nylon	No wash	5	5	5	5	5	5	5	5	5
		40 °C	5	5	5	5	5	5	5	5	5
2,7DHN	Wool	No wash	4	5	3	4/5	4	2/3	5	5	3/4
		40 °C	4/5	5	3	4/5	4	3	5	5	4
	Nylon	No wash	4/5	5	4	4/5	4	4	5	5	4/5
		40 °C	5	5	4	4/5	4/5	4	5	5	4/5
2,5DABS	Wool	No wash	5	5	1/2	5	4/5	5	5	5	5
		40 °C	5	5	1/2	5	4/5	5	5	5	5
	Nylon	No wash	5	5	3/4	5	5	5	5	5	5
		40 °C	5	5	3/4	5	5	5	5	5	5

CA, cellulose acetate; CO, cotton; PA, nylon 6,6; PES, polyester; PAC, acrylic; WO, wool.

Table 14 Colour values of nylon and deaminated nylon fabrics obtained from the laccase catalysis of various aromatic compounds

Compound	pH	Control	Deaminated
1,4DHB	6.0		
		L^* 64.2	L^* 80.2
		a^* 19.6 b^* 13.3	a^* 4.3 b^* 6.7
2,7DHN	8.0		
		L^* 40.9	L^* 41.3
		a^* -2.7 b^* -11.5	a^* -1.2 b^* -17.5
2,5DABS	4.0		
		L^* 65.2	L^* 77.3
		a^* 13.4 b^* 23.9	a^* 7.3 b^* 11.4

laccase-catalysed coloration have overlooked testing for light fastness. Studies by Sun *et al.* [12] and Zhang *et al.* [16] reported that colour fastness of enzymatically dyed fabrics resulted in a higher level of wash fastness for staining and rub fastness but only moderate light fastness. Enaud *et al.* [7] applied an azo anthraquinone dye synthesised with laccase on nylon 6, and reported moderate to poor fastness to light and washing.

Results obtained from colour fastness tests suggest the dyeing properties of all three investigated aromatic compounds vary considerably across both fibre types. In general, the dyed samples have very good resistance against rubbing, mixed levels of resistance to wash fastness and staining, and poor light fastness properties. Post-washing at 40 °C had little effect on the colour fastness results obtained. An understanding of the aforementioned variables, especially the molecular structure of the synthesised coloured products, may give rise to further developments in application processes or finishing treatments to improve fastness properties. Currently, the fixation mechanism is not clear, and further investigations are required to understand how coloured products react with both fibre types.

Effect of the amino groups of fibres on laccase-catalysed coloration

Wool and nylon fibres contain primary amino groups which act as dye sites for acid dyes, therefore acid dyes are applied under acidic conditions, which facilitates the protonation of the amino end groups, enabling the fibre to obtain a positive charge which attracts the acid dye anions by ionic forces, forming salt linkages. If the amino end groups found on wool and nylon fibres are removed, this may provide evidence of how the coloured products generated through laccase catalysis of aromatic compounds may be reacting with each fibre type.

Nylon was selected for investigation because of its simpler structure. Like wool, nylon contains amino end groups; however, nylon contains fewer amino groups than wool and has no side chains, making it a better model for

**Figure 3** Coloration achieved on (a) nylon and (b) wool; and coloured patterns achieved on (c) wool/polyester mixed yarn weave, and (d and e) wool/nylon jacquard weaves, when treated with 1,4DHB in the presence of laccase at various pH values



Figure 4 Coloration achieved on (a) nylon and (b) wool; and coloured patterns achieved on (c) wool/polyester mixed yarn weave, and (d and e) wool/nylon jacquard weaves, when treated with 2,5DABS in the presence of laccase at various pH values

study. Amino end groups were removed from nylon using the Van Slyke method (Scheme 1) forming a deaminated nylon. Control nylon samples were also processed for comparison. The results are presented in Table 14.

Enzymatic dyeing with compounds 1,4DHB and 2,5DABS resulted in only visible staining to a much lighter colour on the deaminated nylon in comparison with control samples. In both cases, control samples dyed to a similar depth of shade as observed previously (Tables 4 and 6). Although coloured compounds were formed in the liquor from laccase catalysis for both aromatic compounds, neither coloured product was able to react with the fibre in the absence of amino end groups, suggesting that primary amine groups were directly involved in the formation of the polymerised colorants through laccase catalysation.

In contrast, enzymatic dyeing with compound 2,7DHN effectively coloured the deaminated nylon to a visibly brighter blue in comparison with the control sample (Table 14). This suggests that coloured compounds generated through laccase catalysis of compound 2,7DHN do not require the presence of amino acids for coloration to take place. However, 2,7DHN has a conjugated structure of two

benzene rings, which should have a higher intermolecular force (consisting of Van der Waals attraction forces) between nylon fibres and coloured compounds catalysed by laccase, especially in the polymerised structure of the colorants.

Design potential

Wool and nylon fibres can be combined and assembled into fabrics for both aesthetic effects and enhancement of functional properties; the inclusion of nylon fibres in blends with wool is particularly popular as it helps improve performance properties such as tensile strength and abrasion resistance. Wool and nylon blended fabrics are usually dyed with a single class of dye, either levelling or supermilling acid, or metal-complex dyes. Solid (where each fibre component is dyed the same hue and depth), and shadow (where each fibre is dyed to a different depth of the same hue) effects are generally more easily obtained with careful selection of a suitable dye and the use of blocking or levelling agents using a one-step dyeing method. However, due to wool and nylon having similar chemical properties and dyeing characteristics, reserve (where one fibre

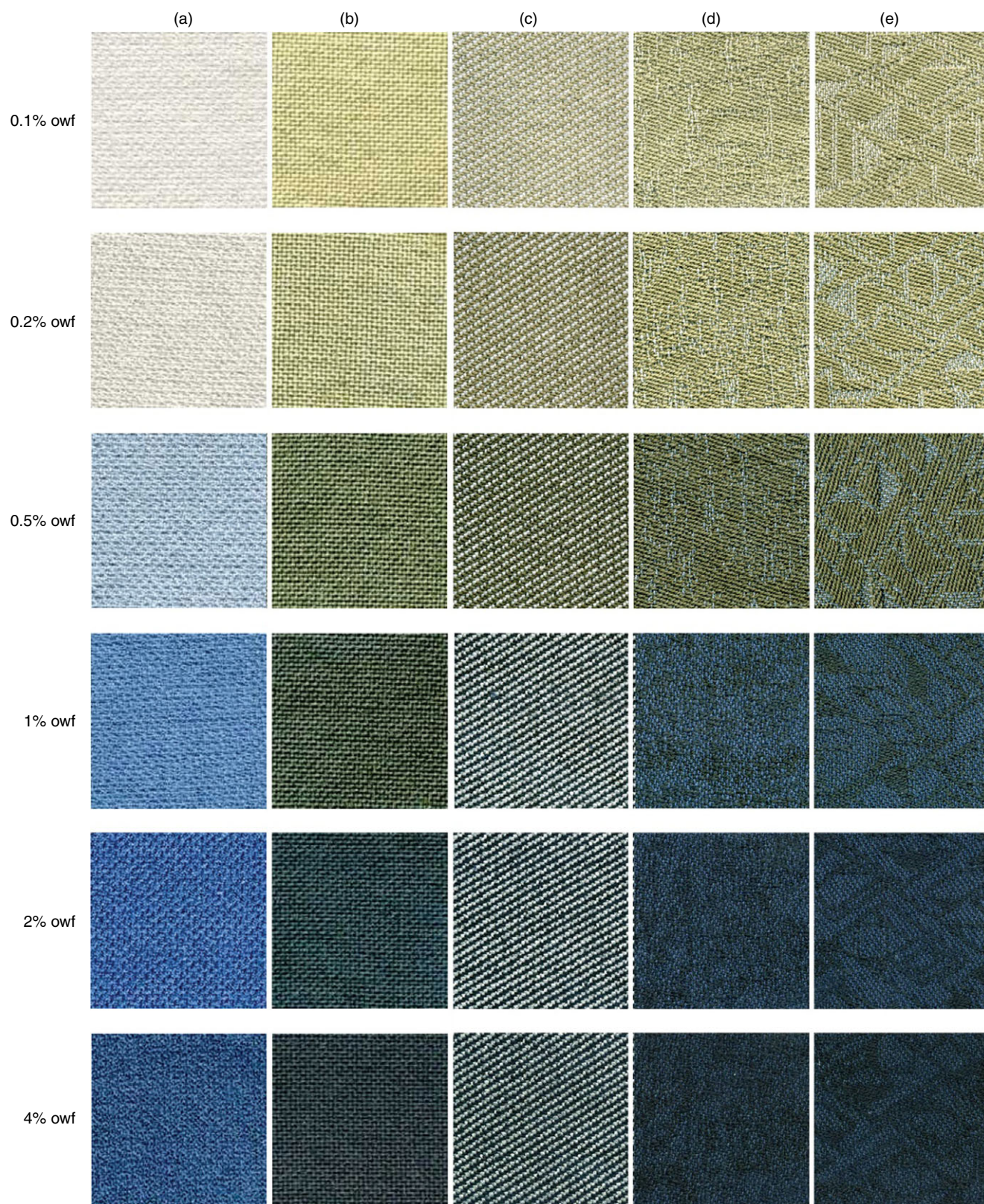


Figure 5 Coloration achieved on (a) nylon and (b) wool; and coloured patterns achieved on (c) wool/polyester mixed yarn weave, and (d and e) wool/nylon jacquard weaves, when treated with 2,7DHN in the presence of laccase at various concentrations

component is dyed, while the other remains undyed), and colour contrast effects (where each fibre component is dyed to a different hue) are difficult to achieve using a single dye and/or a one-step dyeing method, as it is difficult to suppress the dyeability of either fibre, to reserve it to white or dye it another colour. An alternative approach would be to dye each fibre in a separate dyebath using optimal

application conditions in each case to achieve reserve or contrast effects. However, separate processing is often costly and has technical limitations, and therefore wherever possible blends are dyed using a one-step process [33].

The laccase-catalysed coloration process developed could offer a simple and convenient alternative to conventional dyeing processes for the production of either shadow

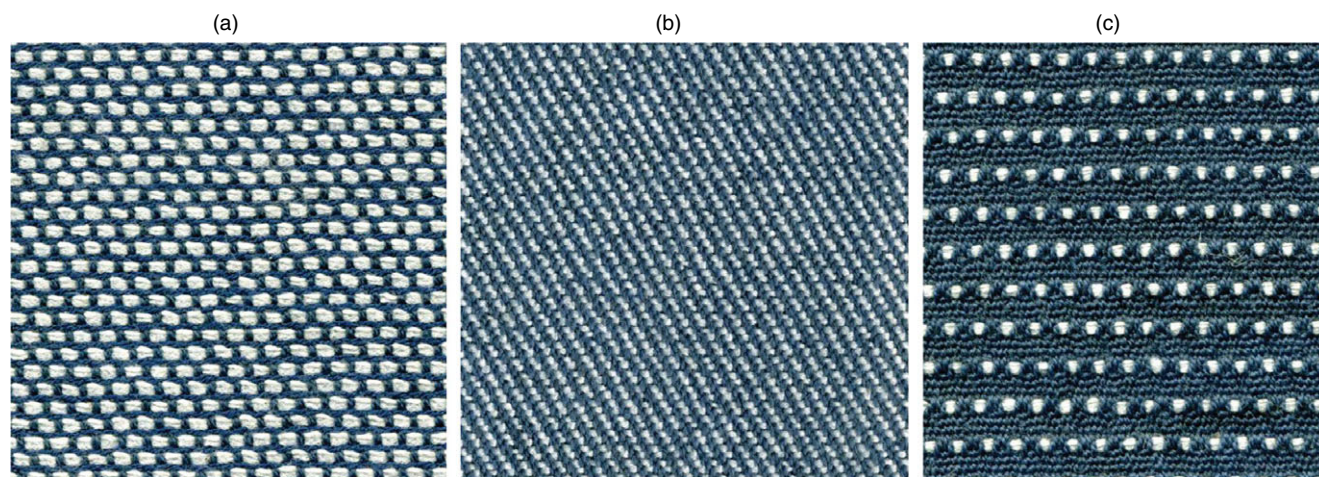


Figure 6 Coloration patterns achieved on (a) wool/nylon/polyethylene, (b) wool/polyester and (c) wool/polyethylene/cotton mixed yarn weaves when treated with 2,7DHN in the presence of laccase at pH 8

or colour contrast effects for wool- and nylon-blended fabrics. This could be further explored with the use of specially constructed fabrics to produce decorative surface patterning. To demonstrate the coloration and design potential by laccase catalysation of the selected aromatic compounds, fabrics were specially constructed using a combination of undyed wool and nylon yarns. Undyed cotton, polyester, and polyethylene yarns were also combined with undyed wool and nylon fibres to illustrate reserve effects. Basic plain, twill, satin, and sateen structures were produced within simple jacquard weaves to generate a selection of woven fabric designs. Fabrics were then dyed by laccase using the one-step coloration process.

Design trials confirmed a wide range of contrast and shadow coloured effects could be achieved on wool/nylon constructed jacquards, and were further enhanced with the exploration of different processing parameters. The exploration of pH values enabled various two-colourway designs to be generated with compounds 1,4DHB (1% omf; Figure 3) and 2,5DABS (4% omf; Figure 4). The latter produced a more diverse colour range. The exploration of different % omf with compound 2,7DHN resulted in a similar array of possibilities: lower concentrations of compound enabled contrasting effects to be achieved, treatment with laccase dyed the wool a green shade and nylon blue. The use of higher concentrations of compound, 2% and 4% omf, resulted in shadow coloured effects, as both fibre types dyed blue after treatment with laccase, nylon dyeing lighter than wool in both cases (Figure 5). Figure 6 demonstrates the possibilities of generating reserve coloured effects through the incorporation of polyester or polyethylene into the blended fabrics; after laccase treatment the synthetic component remained undyed while the wool component was dyed. Variations in jacquard weaves also offer the possibility of new colour tones with an exploration of different weave structures, revealing greater proportions of undyed yarns on show to create the impression of a lighter colour.

Conclusions

The objective of this research was to develop a laccase-catalysed *in-situ* dyeing process for wool and nylon 6,6 fibres as an alternative to conventional dyeing methods.

Emphasis was placed on producing a diverse gamut of colours, which was achieved through the exploration of three different aromatic compounds, a phenol, a benzene and a naphthol derivative, as well as a methodological survey of reaction processing parameters. The use of varied buffer systems, pH values and aromatic compound concentrations proved the most beneficial for increasing the range of possible colours. Previously unreported colours such as pinks, greens and blues were achieved. Colour fastness of the enzymatically dyed wool and nylon fabrics was evaluated, resulting in reasonable good colour fastness to wash, but poor fastness to light. Although the fixation method is not fully understood, the presence of amino end groups were required for coloration of nylon by enzymatic dyeing with 2,5DABS and 1,4DHB, but were not required for coloration of nylon by enzymatic dyeing with 2,7DHN. The key advantages over conventional dyeing methods include the elimination of premanufactured dyes and chemical auxiliaries, and dyeing at ambient temperatures, therefore reducing the complexity of the dyeing process and downstream processing, leading to possible economic and environmental advantages. In addition, the enzymatic dyeing process offers opportunities for multiple colours and shading to be achieved through simple alterations in processing conditions, which is currently not possible with conventional dyes and methods. The results also demonstrate the ability of laccase as a novel and creative tool for the enzymatic process to permit effective surface patterning through controlled applications for shadow and contrast coloured effects. The opportunities discussed could provide the textile industry with realistic and viable options to use enzyme-based surface patterning with the potential of moving towards sustainable development.

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